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The Effect of Calcium to The Absorption Lead In Male Mice (*Mus musculus* L.)

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Abstract: Lead is a poison that can affect human health and is accumulative. Absorption of toxic metals such as lead can be affected by calcium in the body, therefore the calcium contained in food and beverages will increase lead absorption in digestion. The study aims to determine the effect of calcium and magnesium to the absorption of lead in male mice (*Mus musculus* L.).Materials used are Calcium Carbonate, and Lead Acetate. This study used male mice (*Mus musculus* L.) were 60 male mice (*Mus musculus* L.) samples were divided into 10 groups. All groups were given doses of lead at a dose of 40 mg/kg/day, then for group P1 given calcium 25 mg/kg/day, P2 group given calcium 35 mg/kg/day and P3 group was given calcium 45 mg/kg/day. Mice have blood drawn 2 weeks for 3 months. Then the absorption of lead assayed using Atomic Absorption Spectrophotometry air-acetylene flame at a wavelength of 283.3 nm. The data obtained were statistically analyzed by simple linear regression statistical analysis.These results indicate that administration of calcium significantly affect the absorption of lead in male mice (*Mus musculus* L.) of 97%.Based on the above results it can be concluded that the administration of calcium can affect the absorption of lead in mice. With the higher doses will further decrease the absorption of lead.
Keywords: Male mice (*Mus musculus* L.), calcium, lead, Atomic Absorption Spectrophotometer.

Introduction

Lead can interfere with the function of various organ systems of living things, especially for young individuals, lead compounds potentially damage the nervous system so that the children can be accompanied by reduction in intelligence quotient (IQ) that as a result children tend to slow in thinking and unintelligent[1]. Other than that of lead can also cause anemia, damage to the kidneys, and consequently affect the reproductive system birth defects[2].

Some nutrients such as minerals, vitamin B1, vitamin C and vitamin E can affect absorption of lead[3]. In particular the increase of calcium in the diet, especially in children is very important[4]. Children who get more calcium than the recommended amount turns out to have lead levels lower than those who do not get enough calcium deficiency due to lead exposure in children can cause central nervous system disorders[5].

There are also many minerals in drinking water, especially calcium. It takes 3 liters of drinking water for men and 2.2 liters for women per day. At this time, the drinking water is available in packs consist of two groups, namely mineral water and water without minerals. The mineral water is bottled drinking water containing minerals in a certain amount. Mineral water can prevent the absorption of toxic metals such as lead and cadmium in the body[6,7]. Based on the results of the study, the low drinking water minerals such as calcium

and magnesium if consumed in the long term of time will cause health problems such as coronary heart disease, cancer and others[8].

Experimental

Materials

Drinking water without minerals (Amidis), the blood of mice, CMC Na (Carboksi Methyl cellulosa Sodium), the standard solution of lead 1000 mg/mL, perchloric acid 50 %, concentrated sulfuric acid, concentrated nitric acid, Calcium Carbonate, Lead Acetate, Aquadest used as experimental animals drinking water and as a solvent lead acetate, calcium carbonate. Pellets used as animal food.

Methods

Research design

In this study used male mice (Mus musculus L.) aged 6-8 weeks, weighing between 20-40 grams with a healthy condition. Samples were randomly divided into 3 groups, namely groups of P1, P2 and P3. Each group was treated as follows:

P1: Calcium Carbonate 62.5 mg/kgbw/ day (Ca 25 mg/kgbw/day) and Leasd Acetate 40 mg/kgbw/day.
P2: Calcium Carbonate alcium Carbonate 87.5 mg/kgbw/day (Ca 35 mg/kgbw/day) and Lead Acetate 40 mg/kgbw/day.
P3: Calcium Carbonate 112.5 mg/kgbw/day (Ca 45 mg/kgbw/day) and Lead Acetate 40 mg kgbw/day

Preparation of Animal Experiments

Before the animal is given the treatment, the animal must be adapted terkebih advance for a week. Animals (male mice) kept in a sealed plastic enclosure, with the length and width of the enclosure is longer than the animal's body, including the tail, and each cage covered with rice husk. Drinks such as distilled water provided ad libitum. Cages were placed in a room that is not directly exposed to sunlight. cages cleaned and pedestal chaff replaced every two days. Places to eat and drink cleaned and replaced every day. experimental animals are treated in accordance with the ethics committee.

Treatment Animal Experiments

Before the animal is given the treatment, carried out blood sampling for these animals to be used as a control. Then the animal was given treatment for 2 weeks, after completion given the treatment, the experimental group of animals P1, P2, and P3 done taking blood intravenously. Blood sampling performed every 2 weeks for 3 months after treatment. Of each animal taken intravenously 0.15 mL of blood by cutting the tip of the tail of mice to be examined lead levels in the blood of the experimental animals. Animal blood sampling using the restrainer to facilitate the animal handler at the time of cutting the tail.

Preparation of Dose Calcium

Weighed 1 gram of calcium carbonate, was added to the mortar, add 100 ml of water which has to contain CMC Na, and then crushed with a stamper until homogeneous.

Preparation of Solution Lead

Weighed 1 gram of lead acetate, dissolved in 100 mL of water, then homogenized.

Analysis of Lead in Blood Samples

Preparation of Calibration Curve Lead

A total of 1 mL of 1000 ppm lead (the mother liquor) was added to a 100 mL volumetric flask and then added aquabidest right to mark boundaries, the obtained raw lead 10 ug/mL.

Each of 2.5 mL, 5 mL, 10 mL, 20 mL and 40 mL pipette lead standard solution 10 mg/mL in a 100 mL volumetric flask to obtain successive concentration of 0.25 mg/mL; 0.5 mg/mL; 1 mg/mL; 2 mg/mL and 4 mg/mL and measured by flame atomic absorption spectrophotometry at a wavelength of 283.3 nm. Then obtained a calibration curve of lead.

Destruction of Samples

0.15 mL of blood was added 5 mL of concentrated nitric acid and heat on a hot plate at a temperature of 150C for 30 minutes. After it was added with 0.2 ml of 50% perchloric acid and 0.4 ml of concentrated sulfuric acid, then heated in a row at a temperature of 150°C, 200°C and 250°C respectively within 15 minutes. Further heated at a temperature of 320°C for 20 minutes. Lead obtained reduced 6 N Nitric Acid (v/v) with a temperature of 90°C for 30 minutes. Then cooled and diluted with demineralized water[9].

Determination of Lead Levels In Sample

The sample solution that has been prepared with the absorbance measured using flame atomic absorption spectrophotometry at a wavelength of 283.3 nm using Form. Absorbance values obtained should be within the range of the calibration curve of lead standard solution. Levels of lead in the sample is calculated based on the regression equation of the calibration curve.

Statistical Data Analysis

Program observational data were analyzed using linear regression with modest with spss 21 .

Result and Discussion

Calibration curve Lead

Based on the findings of Measurement Calibration curves were measured on metal lead concentration of 0.25 ug/mL, 0.5 mg/mL, 1 mg/mL, 2 mg/mL and 4 mg/mL.

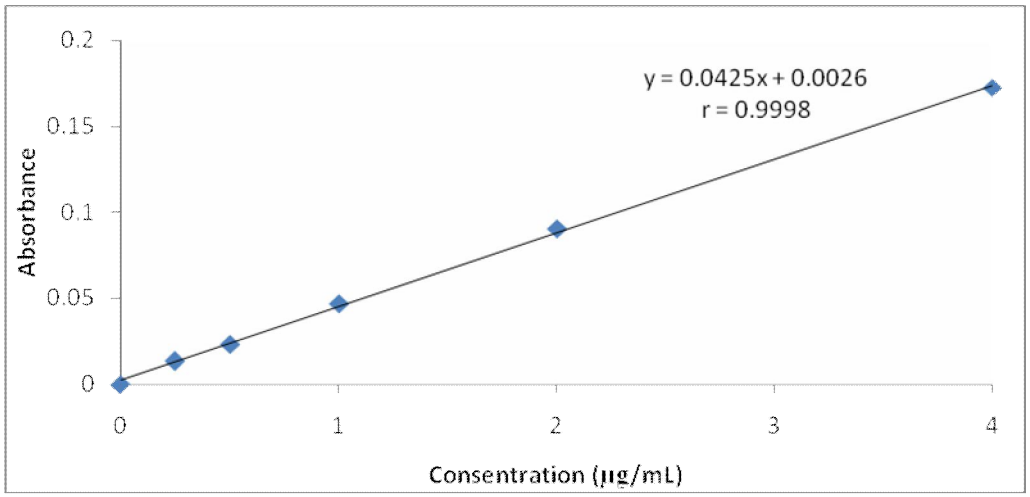

Fig 1.The calibration curve of lead standard solution

The correlation coefficient obtained from the metallic lead can be accepted as the appropriate requirements for the correlation coefficient should not be smaller than 0.995. Coefficient above suggested a linear relationship between the concentration of the metal and absorbance[10].

The Effect of Calcium To The Absorption Lead In male mice (*Mus musculus L.*)

The value of the effect of time and dose levels of calcium on the absorption of lead in male mice (*Mus musculus* L.) can be seen in Table 1. Charts the influence of time and the dose of calcium for absorption of lead in male mice (*Mus musculus* L.) can be seen in Figure2.

Table 1. The Effect of Time and Dose of Calcium on The Absorption of Lead on Male Mice (*Mus musculus* L.)

Consentration of lead in samplel (µg/mL)							
Dose	**Control**	**2 week**	**4 Week**	**6 Week**	**8 Week**	**10 Week**	**Percentage Decrease**
Ca 25	98.071	73.7411*	34.3433*	20.2079*	9.1233*	4.2654*	95.15 %
Ca 35	89.106	46.5482*	26.2681*	16.2074*	8.0636*	3.9351*	96 %
Ca 45	73.991	35.4294*	25.2547*	11.1155*	6.0632*	2.5272*	97 %

Description * = real effect 95% of the control ($P < 0,05$)

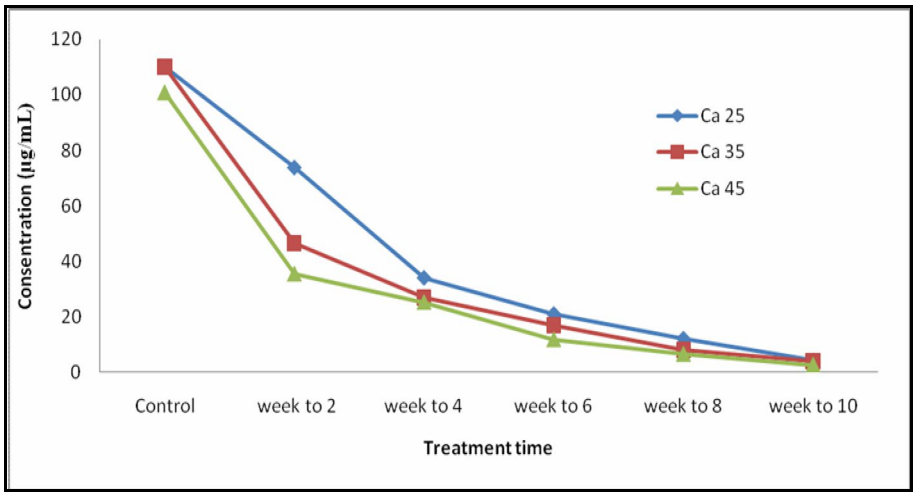

Fig. 2. Effect of calcium on the absorption of lead in male mice (*Mus musculus* L.)

From Table 1 and Figure 2 it can be seen that the lead levels in the treatment group who received calcium at a dose of 25 mg/kgbw/day, 35 mg/kgbw/day and 45 mg/kgbw/day in the same time with the provision of a lead with a dose of 40 mg/kgbw/day of treatment to 2 weeks to 10 weeks visible decrease lead absorption in mice when doses of calcium increased, it is proved that the increase of calcium in the body will decrease the absorption of lead.

This is reinforced by research conducted by previous researchers who explained that the decrease in the concentration of lead in the blood of mice after administration of calcium at a dose of 25 mg/day and 50 mg/day[11]. It is also in line with research conducted by Tordoff who found that monkeys and mice that lack of calcium will increase the concentration of lead in a larger amount compared with the control[12]. The same was done by other researchers who found that increased consumption of calcium given to mice can reduce the absorption of lead in the bones and in breast milk during lactation[5].

Conclusions

The conclusion from this study is :

1. Provision of calcium apparently showing a decrease of 97% against the absorption of lead in male mice (*Mus musculus* L.) .
2. Statistical tests stated that the administration of calcium significantly affect the absorption of lead in male mice (*Mus musculus*.L.).

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